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AN APPARATUS FOR CONSTANT PRESSURE PERFUSION OF ORGANS

The apparatus described herein provides a means of supplying an organ with heparinized blood, or other perfusion fluid, at a constant pressure regardless of the opposing resistance encountered in the organ under study.

The problem originally in mind when the pump was designed was the study of the kidney under conditions of constant blood pressures. Otherwise the kidney would remain as nearly as possible in its natural functional condition, its innervation preserved,and its relations undisturbed. Other problems, such as coronary perfusion, continuous cross transfusion, liver perfusion, and blood flow may be suited to its characteristics. That is, it holds one variable, blood pressure, constant while the effects of varying others are studied.

RESUME OF PERFUSION APPARATUS

The technique of perfusion has long been of exceeding interest to men of medical sciences. Belt, Smith, and Whipple[1] in a comprehensive study on the factors concerned in perfusion of living organs, lists among the pioneers in the field: Le Gallois, for his early statement of the possibility that by perfusion, part or parts of the body could be kept alive indefinitely; Ludwig and Schmidt, for their apparatus that supplied a constant perfusion pressure by forcing the perfusate out of a reservoir; Fry and Gruber, for the development of a mechanical means of producing pulsating pressure and for the development of an artificial aeration device; Jacobj, for his apparatus that produced pulsatile perfusion pressure by rhythmical compression of a balloon placed in the arterial side of the circulatory system; and Brodie, for perfusing an organ, using no other blood than that obtained from the animal itself, by placing a length of distensible rubber tubing in the arterial side of the circulatory system and alternately compressing and releasing it with a wooden arm.

Artificial perfusion is not the only means that has been used in the study of organs. By use of the heart-lung preparation after the method of Knowlton and Starling, modified with a T tube on the arterial side as a tap to a kidney, Bainbridge and Evans[2] were able to demonstrate that the perfused kidney was able to secrete urine. Verney and Starling[3] used two dogs to prepare a heart-lung-kidney experiment. One dog was used in the heart-lung system, the other furnished the kidney. This preparation was used for studies on isolated kidney secretion.

One of the simplest methods of obtaining a force to cause the perfusion fluid to circulate in a system and to supply an organ is one that has already been mentioned, i.e., alternate compression of a rubber tube or a rubber bulb. Kleinberg, Gordon, and Charipper[4] used the force from a bulb to cause pulsations in a rigid tube containing the perfusion fluid. The tube was fitted with inlet and outlet valves so that when the bulb was compressed the fluid was forced out, when the bulb was released the fluid was able to refill the tube. The valves were of a gravity type that relied on backflow to actuate them.

For the perfusion of organs from small animals Long[5] built a rather elaborate glass and rubber pump, the force being supplied by a rubber bulb that was compressed by means of an electromagnet.

Because of its simplicity and the readiness with which it lends itself to demonstration, several other models of circulatory systems have been constructed around a "heart" consisting of rubber bulbs and a means of compressing them[6,7,8].

Valves in perfusion systems have always been a source of concern. A means of perfusion with a valveless system makes use of a set of rollers on a pulley. These rollers are allowed to pass along a length of rubber tubing, compressing it progressively along its length and driving ahead fluid that is in the tubing. Burns[9] describes a pump of this nature that utilizes three rollers mounted on a pulley. The rubber tube is held in a circular position by grooved blocks. Another investigator[10] used a rheostat controlled motor to drive an offset roller over a horseshoe shaped tube. The pressure of the roller on the tube was adjustable, thereby affording a means of regulating the force of fluid propulsion.

Unique, because of its ability to perfuse coagulable blood, is the mechanical heart devised by Brull[11]. The heart is made from the aorta of a 15-30 kg. dog. It is constructed in such a way that all the surfaces which may contact the blood are a part of this aorta. Propulsion of the blood is by means of a roller-pulley system such as that described above. The aorta is protected in the region over which the rollers pass by a length of rubber tubing. By utilizing the branches of the aorta, several sources of blood may be tapped, e.g. auricular blood, arterial blood, or perfusion fluid from a reservoir. Pressure is regulated by a shunt system that is coated on the inside by the carotid artery. Pulse rate is controllable by the speed at which the pulley is revolved, and systolic pressure is adjusted by the pressure allowed the rollers to exert on the aorta.

Rostorfer, Edwards, and Murlin[12] employed a syringe type pump fitted with inlet and outlet valves of the glass float type and powered by a motor-driven eccentric in their liver perfusion experiments. An artificial aeration apparatus was used in conjunction with the pump.

In an attempt to produce a system that would provide a constant flow, Richards and Drinker[13] used the reciprocating type pump. This was a double pump, one-half providing flow through an artificial aeration device, the other perfusing the organ. They employed a cam-driven rocker arm, the length of which was adjustable, thereby determining the stroke output of the pump. With a constant speed and a constant stroke output the volume output per unit of time would remain constant. The valves consisted of lengths of rubber tubing that were compressed by wooden spades. A cam and pushrod system, driven by a chain and sprocket from the same shaft as the rocker arm cam, actuated the valve system. Richards and Drinker achieved their purpose of constant outflow except for the valves. The distensibility of the short rubber tubes would induce a source of error, the flow being slightly less under conditions of high pressure than under conditions of low pressure.

Hooker[14] believed that a pulsatile pressure was necessary for proper perfusion. In order to reproduce the pulse wave form that is naturally encountered, he utilized the principle that the form of the wave is dependent upon the velocity of the piston. The crank throw that transmitted the reciprocating motion to the piston was mounted on a movable carriage. A cam on the shaft operated against a fixed surface so that as the cam rotated, the cam, shaft, and crank throw moved forward or backward according to the shape of the cam. The result of the carriage motion and the motion afforded the piston by the crank throw determined the velocity of the piston. The shape of the cams, therefore, determined the shape of the pulse wave. The system made use of two pumps with a common output tube. They were so timed that upon the completion of the systolic stroke of one, the other was just beginning its systolic stroke. This achieved an almost continuous outflow which, as Hooker admits, is in variation with natural cardiovascular flow. For the purpose of altering pulse pressure he made use of two pinch clamps. One of these was placed on a by-pass line, the other immediately before the organ being perfused. The first served to control mean pressure, the second was said to have three functions: (1) to lower the mean pressure, (2) to lower the maximum pressure, (3) to raise the minimum pressure. These statements being true, then, by proper adjustment of the two pinch clamps pulse pressure alone could be varied.

A pump used by a number of investigators[15,16,17] is the Dale Schuster[18] perfusion pump. This is another double pump that supplies an aeration device as well as the organ that is being perfused. The two pumps operate off the same shaft, but each is instantly and independently adjustable. The pump uses a diaphragm that is moved up and down by a pushrod, rocker arm, and crank throw arrangement. Placed over the diaphragm is a glass funnel that has a rubber finger stall fastened to its tube. The funnel and the stall are filled with a fluid. Through this fluid medium movement of the diaphragm is transferred to the stall causing it to inflate and deflate. Over the stall is placed a glass dome fitted with an inlet valve and an outlet valve which govern the direction of flow through the dome. Upon inflation of the stall, fluid inside the dome is driven out through the outlet valve; conversely, upon deflation of the stall, fluid is pulled into the dome through the inlet valve. Output pressure of the pump is controlled by the extent of movement imparted to the diaphragm. This extent of movement is governed by a screw adjustment which changes the position of the rocker arm fulcrum. Stroke rate is controlled by shaft speed.

A similar type pump employing an electrically driven ten cc. syringe to inflate the finger stall was used by Whittaker and Winton[19] in experiments to determine the apparent viscosity of blood flowing in the hind limb of the dog. Later it was used by Murray, Delorme, and Thomas[20] in their artificial kidney.

Pappenheimer and Soto-Rivera[21] used a bellows to inflate the finger stall in a pump used for determination of effective osmotic pressures of plasma proteins.

An interesting type pump described by Palmer[22] is very simple in construction and requires materials that may readily be found in every laboratory. The system consists of a glass cylinder one inch in diameter and six inches long, three one-inch rubber stoppers, a toy balloon, rubber dental dam, a glass bottle, and a few short lengths of glass tubing. Valves for the system are made by lightly stretching dental dam across the end of a glass tube that projects through a rubber stopper. The inlet valve will be inside the glass cylinder, and the outlet valve will be inside the bottle which serves as a reservoir. The balloon is placed over the stopper through which the inlet tube passes so that when it is filled by fluid, its bottom covers the end of a glass tube projecting through a stopper in the lower end of the glass cylinder. A second tube passing through the bottom stopper is connected to a source of compressed air. When the balloon is filled, compressed air is allowed to enter the cylinder. The air forces the fluid in the balloon out through the outlet valve and raises the balloon off of the tube that it covered. This allows the compressed air to escape and the balloon to be refilled through the inlet valve. With the proper adjustment of the compressed air, the cycle is automatically repeated. A modification of this pump by Lazier[23] has a greatly increased output.

Paff, Rubin, and Hamilton[24] constructed an ingenious pump consisting of a glass cylinder having a side arm, a tube of plastic that fits inside, and two rubber stoppers fitted with glass tubes that contain glass valves. The plastic tube is held in place inside the glass cylinder by folding its ends back over the ends of the cylinder and inserting the stoppers. A pulsating air source such as is obtained from the Carrel-Lindbergh[25] valve is connected to the side arm. The force of the air compresses the plastic tube and forces the blood out through the outlet valve. During the interval between pulsations the tube is allowed to fill through the inlet valve. In Warburg tissue cultures, the plastic used, "Voltron", exhibited no toxicity. Systolic pressures as high as 300 mm. Hg. have been obtained from this pump.

Electromagnetic force was used by Rosenberger[26] to move a piston in a cylinder to pump fluid. The pump is so constructed that the fluid comes in contact only with glass. A glass cylinder having a carefully ground glass valve in its lower end is fitted with a double walled glass piston that has a similar valve in its lower end. Sealed between the two walls of the piston is a cylinder of soft iron. Around the outside of the cylinder, over the area of the iron cylinder, is placed two solenoid coils. Both solenoids are connected in separate circuits through a three pole automatic mercury switch that is mounted on a rocking device. By this arrangement they are switched on and off one after the other with an intermediate period during which both are on. This moves the magnetic field up and down. Therefore, the piston, tending to remain in the center of the field, moves in a like manner. As the piston moves up, the valve in the cylinder opens and allows fluid to pass up into the cylinder; as the piston moves down, the valve in the cylinder closes, the one in the piston opens, and fluid is forced out of the pump.

A pump described by Allen[27] uses water suction as a source of force for operating the system. A glass inlet from the suction device passes through a drying chamber to a glass reservoir having a capacity of 100 cc. From the reservoir two glass tubes pass to the organ chamber. The tube from the bottom has a stopcock immediately after it leaves the reservoir.

Thence, through one arm of a Y tube, blood passes into the organ chamber and the cannula to the organ. The second arm of the Y tube is open to atmospheric pressure through a stopcock. The other glass tube passing out of the reservoir leaves near the top and connects with the bottom of the organ chamber. From the top of the organ chamber is another outlet to atmospheric pressure through a stopcock. The system operates in the following manner: the reservoir is filled with the perfusion fluid, and hydrostatic pressure causes it to flow out through the bottom tube to the organ. From the veins of the organ the fluid flows out into the chamber and collects in its bottom. Suction is applied, creating a partial vacuum in the reservoir which pulls the collected fluid up through the other tube back into the reservoir. In late models a stopcock has been added to the bottom of the organ chamber for the purpose of obtaining samples. Also, a small centrifugal pump has been added to the arterial tube prior to entering the organ chamber. The main application of this pump has been in apparatus used for the culture of tissues.

Probably the best known perfusion apparatus for organ culture is the Lindbergh pump[25]. It is an elaborate system of glass having the ability to supply a pulsating flow of fluid to an organ while maintaining aseptic conditions throughout. In principle of operation it is relatively simple. An adjustable rotary valve is used to supply a pulsation source of air pressure which is transmitted through a column of oil to a gaseous medium in the sterile chamber. This gaseous medium transfers its pulsations to the perfusion fluid. The pulsations of the fluid are directed by a pair of valves to produce a pulsating flow of fluid.

One of Lindbergh's earlier pumps[28] is reminiscent of a pump devised by Archimedes. It depended upon a head of hydrostatic pressure for its source of perfusion pressure. This head was maintained by causing the fluid after passing through the organ to be returned to the reservoir by progression up a coil,held on the oblique,by revolving the entire apparatus while preventing its rotation.

The pumps described above will give an idea of the many and varied principles that have been applied in devising apparatus for studying the function of organs by the technic of perfusion.

DESCRIPTION OF THE CONSTANT PRESSURE PERFUSION PUMP

Absolute control of pressure can be obtained very easily by means of electronic control circuits. A mercury manometer affords a relatively simple device for measuring mean pressures and automatically maintaining predetermined pressure. Compressed air is a convenient controllable source of force for providing perfusion pressure. Positive-action type valves do not rely on backflow for actuation. It was with these principles in mind that this pump has been designed.

The basis of the pump is a double solenoid valve, the component parts of which are shown in figure 1. The air valve portion is constructed of a section of brass tubing 1 inch in diameter and 4 5/8 inches long, (D). A steel plug (B), turned with a shoulder on its upper end, and a 60° valve seat (C), 1/4 inch in diameter opening through a 1/8 inch hole to atmospheric pressure on its under side, is soldered into the upper end of the brass tube. Inside the tube is fitted a steel plunger (F), 2 5/8 inches long, that slides easily yet has a minimum of lateral displacement. This plunger has a valve (E), turned on its upper end that fits the valve seat (C). On its lower end is a similar valve (I) that is on an extension 1/4 inch in diameter and 1/4 inch long. The plunger has slots (G) lengthwise

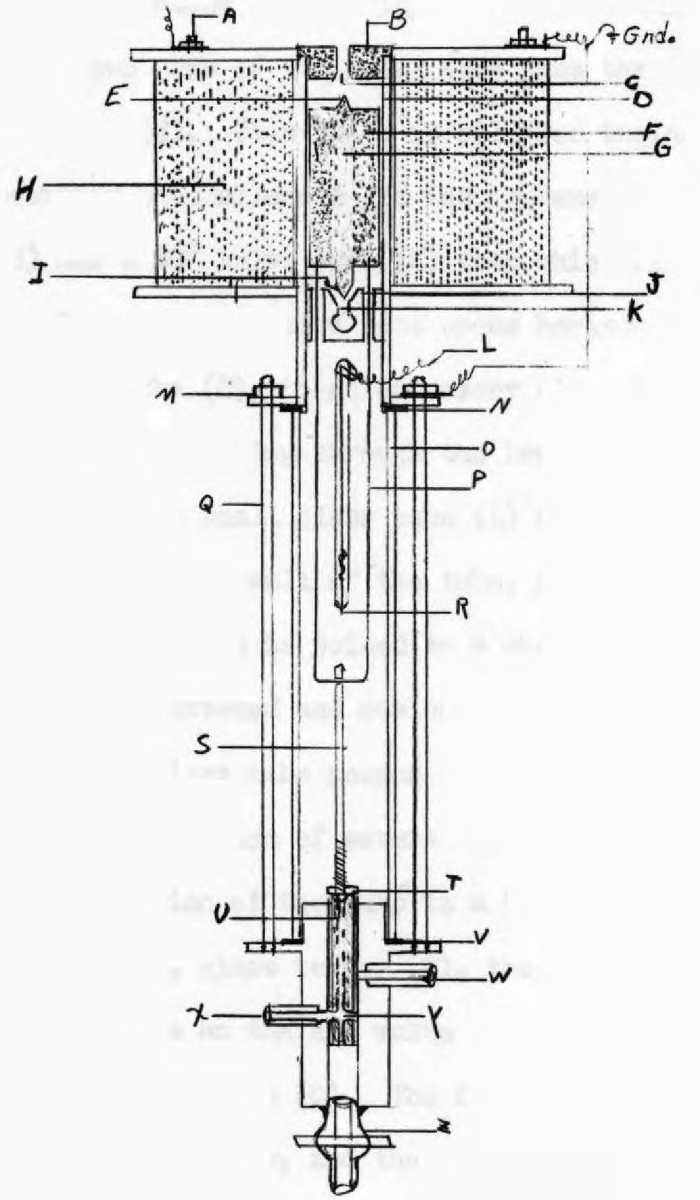

Figure 1. Schematic Sectional Diagram of the Air-Blood Solenoid Valve

to facilitate the passage of air past it. Into two small holes, drilled lateral to the lower valve, are fitted the two ends of a U shaped 20-gauge stainless steel tube, the loop of the U having been flattened. The two arms of the steel tube pass through guide holes in the brass block (J). This block is soldered inside the brass tube in such a position that allows a 3/8 inch movement of the plunger. The block (J) has a 60° valve seat (K) into which fits the lower valve of the plunger. The valve seat opens horizontally through the side of the brass tube (D), to an accessory air solenoid valve (SV_1, see figure 2). Passing through the brass tube immediately below the block (K) is a small glass tube (L) containing a copper wire. After passing through the wall of the tube, it bends 90° and extends downward. The copper wire is joined to a short length of platinum wire that has been flattened and sealed in the end of the glass tube. The point where the glass tube passes through the brass tube is made air-tight by the application of several coats of cement.

The chamber portion of the pump is a 5 5/16 inch length of 1 inch, inside diameter, glass tubing (O), the ends of which have been ground flat. A flange on the air valve and one on the fluid valve are joined by two brass rods (Q). The flange on the fluid valve is tapped to take a 10-32 screw, and the ends of the brass rods are threaded to fit. The flange on the air valve is drilled to allow free passage of the rods. Gaskets (N,V) are placed between the flanges and the glass tube. Nuts and lock washers (M) are tightened down on the upper ends of the rods. This pulls the ends of the glass tubing firmly against the gaskets, holds the three portions of the pump together, and insures air-tight junctions between the two valves and the glass chamber.

The fluid valve portion of the pump is constructed from a 2 1/16 length of brass rod, 1 inch in diameter. Through its center a 1/4 inch hole is drilled and reamed by hand to insure a smooth surface. The flange described above is soldered in place, 3/8 inch below the top surface. This allows the brass cylinder to extend into the lumen of the glass chamber and serve as a guide to keep the component parts in alignment. On one side, 3/4 inch from the top, a hole 1/8 inch in diameter is drilled through the wall and into the lumen of the cylinder. This is counter-bored to 5/32 inch for a distance short of the lumen by 1/8 inch. Into this hole a 1/2 inch length of 1/8 inch, inside diameter, brass tubing (W) is inserted and soldered in place. Below this tube, 3/8 inch on the opposite side, a similar tube (X) is affixed. These two tubes constitute the outlet and inlet, respectively, of the fluid valve. A small metal stopcock (Z) is soldered in the lower end of the brass cylinder.

A brass slide valve (U) is turned to a smooth finish and to such dimensions that it will slide easily within the cylinder but will allow no leakage. The upper end of the slide is drilled and tapped to take a 4-36 screw. A small brass rod (S) is threaded on one end to fit. A locking nut (T) is screwed on the rod and then the slide is screwed tightly against it. By this means the slide valve may be adjusted to the optimum position in relation to the inlet and outlet tubes. The upper end of the small brass rod (S) has a hole drilled through it. Through this hole passes the flattened loop of the stainless steel tubing (P). This links the air valve with the fluid valve.

Over the top of the brass cylinder (D) is slipped the solenoid coil (H). The coil consists of approximately 10,200 turns of 38-gauge enameled copper wire. The top of the coil is made so that it will slip down over the brass tube only as far as its proper position.

Figure 2 is a schematic representation of the entire system. Immediately preceeding the air portion of the double solenoid valve is an accessory air solenoid valve (SV_1).

The fluid outlet tube from the pump leads into a one-liter pressure bottle that serves as a reservoir (R). From the reservoir is the outlet tube that leads to the organ being perfused. The pressure control manometer (M) is also connected into the system at this point.

The manometer is the ordinary U tube mercury manometer with a platinum electrode sealed in the wall just above the curved portion of the loop. A ten-inch length of small bore glass tubing that has a platinum electrode sealed in its lower end and connected inside the tube to a length of small copper wire forms the other contact. The end of the electrode may be placed inside the manometer tube at any desired height above the zero level of the mercury column and held in that position by a clamp.

The entire apparatus is housed inside a wooden box with a sliding glass front. Two 60-watt light bulbs placed near the floor of the box furnish heat enough to keep the apparatus at 37°C. The bulbs may be turned on or off at will by a switch. A thermometer (T) is suspended from the top of the box to indicate the temperature of the system.

The electronic control circuit, illustrated in figure 3, is built on a separate chassis eight inches square. It is connected to the pump apparatus by an eight-wire cable with octal connectors (P_m, P_f).

OPERATION OF THE PERFUSION APPARATUS

Figure 4 is a schematic representation of the apparatus under operating conditions. The blood inlet (x) is connected to the arterial side of the animal's circulation. A source of compressed air is connected to the

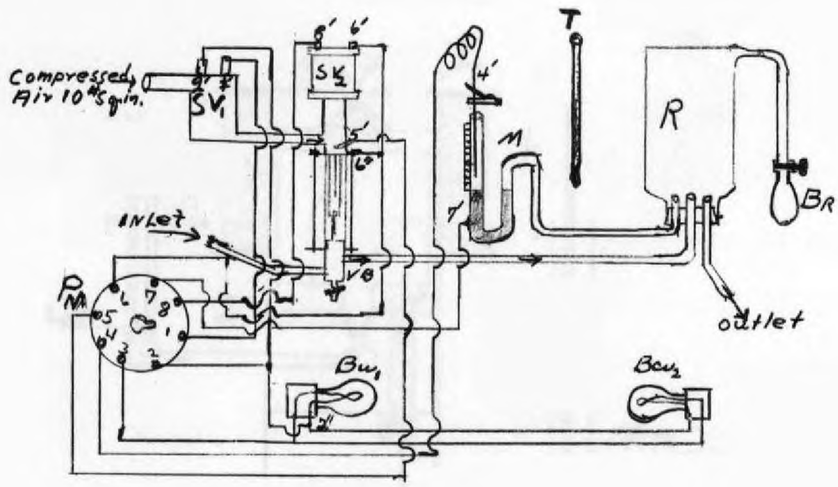

Figure 2. Schematic Diagram of the Perfusion Apparatus

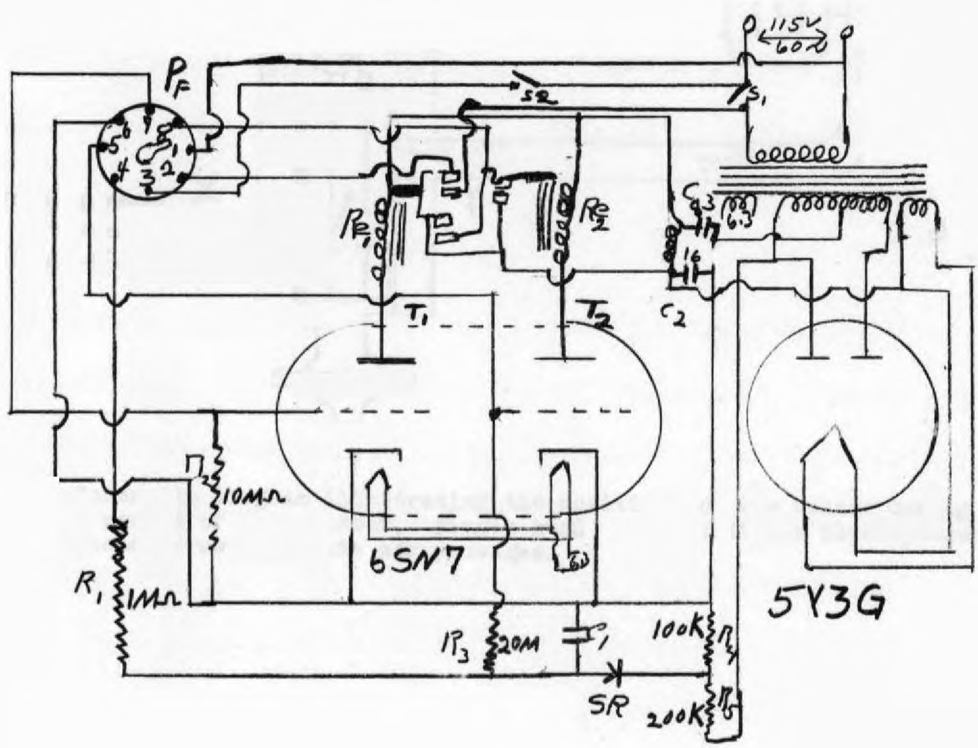

Figure 3. Circuit Diagram of the Electronic Control Circuit

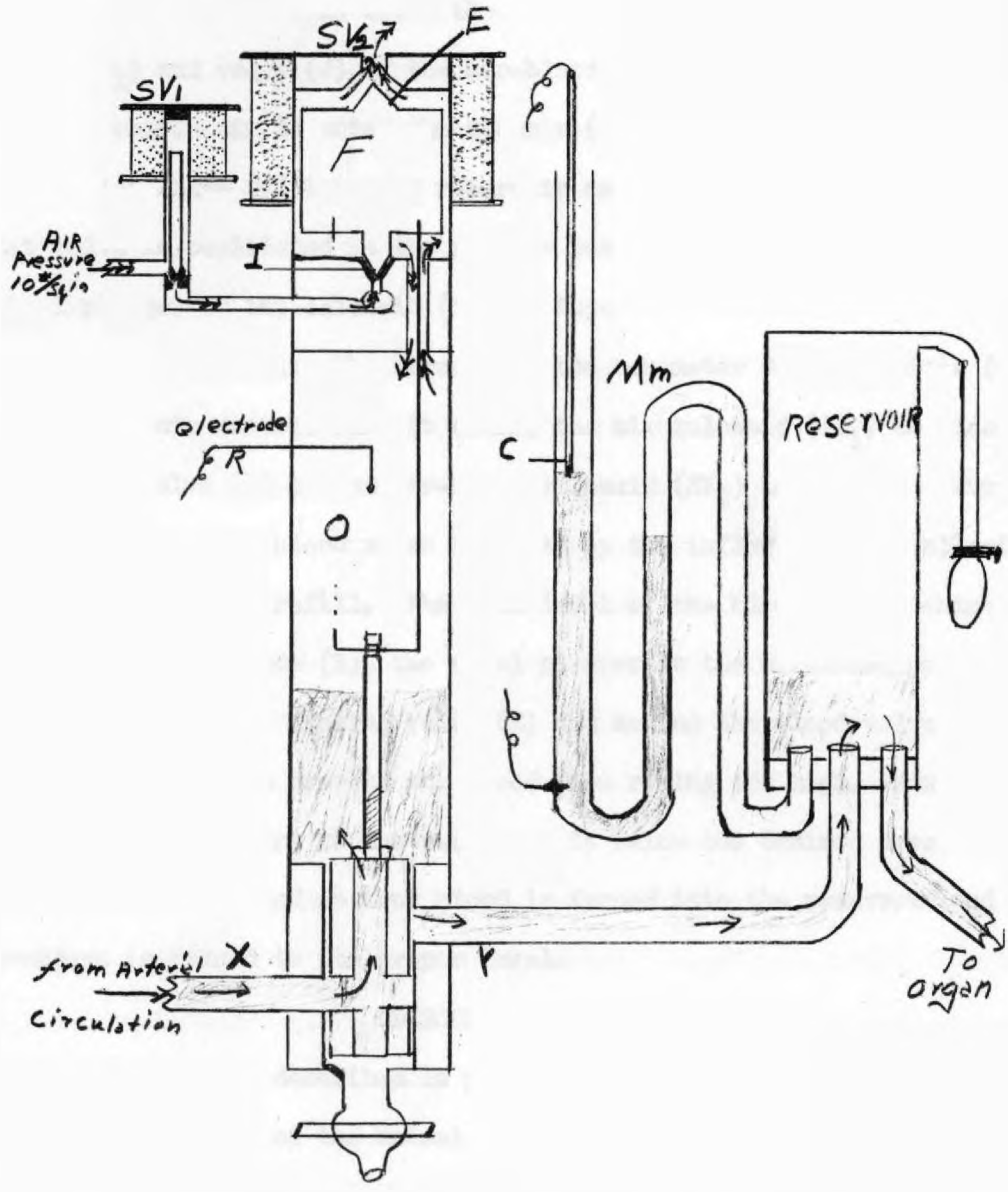

Figure 4. Schematic diagram illustrating the positions of the valves during the filling period of the chamber. Single head arrows indicate blood passages; double head arrows indicate air passages.

accessory air solenoid valve (SV_1) at the place indicated. When the pressure in the reservoir is below the desired pressure, accessory air solenoid (SV_1) and valve (J) of the air-blood solenoid (SV_2) are open allowing compressed air to enter the chamber (O) and force the blood through the outlet valve (y) into the reservoir and on to the organ. The outlet valve is positioned in the outflow position by its linkage with the steel plunger in the solenoid (SV_2). When the pressure in the reservoir is sufficient to raise the mercury in the manometer to the contact (c), the electronic control circuit causes the air solenoid (SV_1) to close. Likewise, valve (I) of the air-blood solenoid (SV_2) is closed, valve (E) is opened, and the blood valve is moved up the inflow position allowing the chamber (O) to refill. When the level of the blood in the chamber reaches the electrode (R), the steel plunger in the air-blood solenoid valve will move up, closing valve (E) and moving the blood valve to the outflow position to prevent the blood from rising too high. However, at any time the pressure in the reservoir is below the desired pressure, the valves are positioned so that blood is forced into the reservoir and the pressure is raised to its proper level.

DISCUSSION

The apparatus described is not intended to replace the heart in the circulatory system of the animal under investigation. Rather, it is more of a second stage pump placed in series with the heart so that regardless of systemic blood pressure, the pressure delivered to the perfused organ will always remain constant. Consequently, the animal's heart and lungs remain integral parts of the circulatory system to the perfused organ.

Though not a pulsatile pump in the sense that it accurately reproduces the pulse waves normally encountered in the circulatory system, the system does supply a pulsating pressure. The initial surge of force caused by the compressed air gives a slight rise in pressure above the mean pressure, this corresponding to systole. The inertia of the mercury column allows the pressure to drop a slight amount below the mean pressure before the contact in the manometer is broken. This may be favorably compared to diastole. The large air cushion in the reservoir absorbs much of the surge as does the aorta in the actual systemic circulatory system.

By virtue of its design the entire system is of rugged construction. There is no deteriation of rubber bulbs to demand frequent replacement. Galli-Mainini[10] states that the life of the rubber tube in the roller type pump is about four days. Therefore, it is reasonable to assume that the aorta used by Brull[11] will have a useful life much less than this. A further disadvantage to the Brull heart is the drastic surgery involved in the removal of the aorta. Pumps of this type when operating against high pressures would be subject to large back leakages.

A great number of the systems employ valves of the gravity or float type. These require movement of the fluid medium to function. To prevent leakage these valves must be accurately ground and fitted. Furthermore, even in heparinized blood, there is a tendency for a slight amount of fibrin formation. This naturally centers about the valves because of turbulence, and sludge that forms elsewhere in the system tends eventually to collect in the valves. Very small amounts can cause improper fitting of the surfaces, and great leakages result. With the positive action type valves used in the apparatus described herein, backflow is not needed to actuate them. Instead, the force of the solenoid and the weight of the iron plunger move the slide automatically with each cycle of the pump.

Systems that employ the shunt type of pressure control depend upon the ability of the pump to have an output in excess of that needed. They function by preventing excess pressure and make no provision for increasing the pressure when it is below this pressure. By using the combination of a mercury manometer and an electronic control circuit such as the one shown in figure 3, both the prevention of excessive pressures and the increasing of low pressures may be accomplished. The mean perfusion pressure is thereby automatically maintained at the desired level.

Because of the accumulation of electrolytic products about electrodes in blood, even under conditions of small current flow, the electronic control circuit had to be one that was actuated by a minimum of current flow between its electrodes. The use of high resistance grid leak resistors (R_2, 10 Meg ohms, R_3, 20 Meg ohms, in figure 3) limits this current flow. In operation this method is perfectly satisfactory. No accumulation of any sort has been noted on the platinum electrode (E, in figure 1) after an hour of operation in a medium of blood.

Fluid flow up to 250 cc. per minute has been obtained with a pressure output of 200 mm. mercury pressure by the system. Pressures much higher than this have been obtained with a slight decrease in fluid flow. The limiting factor in output is the time available for filling the pump chamber. Obviously, it is not possible to obtain an outflow greater than the inflow. This filling time can undoubtedly be shortened by increasing the diameter of the inflow tubes if such a need arises.

As experience is gained in the use of this pump, various other improvements are being made, and still others are being planned for use in future models. Among these are the use of rotary type valves in place of the slide type, the addition of thermostatic temperature control, the incorporation of a modified Ludwig stromuhr for rate of flow determinations, and adaptations to permit the use of other measuring devices for the photoelectric determination of per cent oxygenation of both arterial and venous blood so that the metabolic rate of the organ may be determined continuously.

It is earnestly hoped that by controlling as many physical variables as possible a greater knowledge of the functions of organs such as the kidney may be gained.

SUMMARY

1. A brief history of the technique of perfusion has been presented along with several outstanding pieces of apparatus for perfusion used by other investigators.

2. An apparatus has been described that permits blood pressure to be maintained at a constant value, thereby eliminating the effect of blood pressure during the course of an experiment.

3. The apparatus provides a flow of perfusion fluid up to 250 cc. per minute at an output pressure as great as 200 mm. mercury pressure. Greater output pressures are possible with a slight reduction in flow.

4. The apparatus functions automatically for indefinite periods, requires little attention during operation, and is easily adjustable while in operation.

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